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Molecular analyses reveal a new species in *Melanoderma* from tropical China

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ABSTRACT — A new wood-decaying polypore, *Melanoderma disciforme*, is described from tropical China based on morphological characteristics and rDNA ITS sequences. The new species is characterized by perennial effuse-reflexed basidiocarps, a narrow pileus with a thin black crust on the pileal surface, a dimitic hyphal system with dextrinoid and cyanophilous skeletal hyphae, and the presence of fusiform cystidioles and cylindrical basidiospores. Molecular analyses indicate that the new species and *M. microcarpum* cluster together with full support.

KEY WORDS —*Basidiomycota*, *Polyporaceae*, phylogeny, taxonomy

Introduction

The genus *Melanoderma* B.K. Cui & Y.C. Dai was recently established to accommodate the newly described species *M. microcarpum* B.K. Cui & Y.C. Dai (Cui et al. 2011). The genus is characterized by perennial pileate to effuse-reflexed basidiocarps, a black pileal surface with a thin crust, a dimitic hyphal system with dextrinoid cyanophilous skeletal hyphae, apically encrusted cystidia, and the presence of cystidioles and cylindrical thin-walled basidiospores. Microscopically, *Melanoderma* resembles *Perenniporia* Murrill in having dextrinoid and cyanophilous skeletal hyphae. However, *Melanoderma* is distinguished from *Perenniporia* by its distinctly thin-walled, non-dextrinoid, and acyanophilous basidiospores (Cui et al. 2011).

China is very rich in wood-inhabiting fungi species, and recently extensive studies have been conducted on species diversity, taxonomy, ecological patterns, and phylogeny of wood-inhabiting fungi (Dai 2010, 2011, 2012, Dai et al. 2007, Ma et al. 2011, Zhang et al. 2009, Zhou & Dai 2012). Two polypore specimens collected in 2005 from a tropical forest of Yunnan Province were subsequently identified and deposited as *Melanoderma microcarpum*. But recent comprehensive morphological and molecular analyses suggest that these specimens represent an undescribed *Melanoderma* species. Here we clarify its taxonomic status and describe it as a new species.

Materials & methods

Morphological studies

The specimens studied are deposited in the herbarium of the Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, China (IFP). The microscopic procedure follows He & Dai (2012). Sections were studied at magnifications up to ×1000 using a Nikon Eclipse 80i microscope and phase contrast illumination. Measurements were made from sections stained with CB. In presenting basidiospore size variation, the 5% of the measurements excluded from each end of the range are shown in parentheses. The meanings of abbreviations are as follows: CB = cotton blue, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = negative in Melzer's reagent, KOH = 5% potassium hydroxide, L = mean basidiospore length (arithmetic average of all basidiospores), W = mean basidiospore width (arithmetic average of all basidiospores), Q = range of L/W ratios from individual specimens, and n = number of basidiospores measured from given number of specimens. Special color terms follow Petersen (1996).

Phylogenetic analysis

Total DNA was extracted from specimens using Phire® Plant Direct PCR Kit (Finnzymes, Finland). Primers ITS5 and ITS4 (White et al. 1990) were used to amplify ITS sequences following PCR procedure set forth by Stöger et al. (2006). The DNA was sequenced at Beijing Genomics Institute. The most similar sequences were downloaded from GenBank NCBI (http://www.ncbi.nlm.gov) using the BLAST tool. Sequences were aligned using ClustalX (Thomson et al. 1997) and manually edited to allow maximum alignment and minimize gaps. Maximum parsimony and Bayesian analysis were applied to the ITS dataset. All characters were weighted and gaps were treated as missing data. Maximum parsimony analysis were carried in PAUP* (version 4.0b10; Swofford 2002). Trees were inferred using the heuristic search option with TBR branch swapping and 1,000 random sequence additions. Max-trees were set to 5000 and no-increase, branches of zero length were collapsed, and all parsimonious trees were saved. Nodal support was tested with bootstrap of 1000 replicates using the heuristic search option (TBR and MULTREES options on) (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for all trees generated under different optimality criteria. Bayesian analysis was carried in MrBayes3.1.2 (Ronquist & Huelsenbeck 2003) and models of evolution were identified using MrMODELTEST2.3 (Posada & Crandall 1998, Nylander 2004). Four simultaneous Markov chains were run starting from random trees and keeping one tree every 100th generation until the average standard deviation of split frequencies was below 0.01. The value of burn-in was set to discard 25% of trees when calculating the posterior probabilities. Bayesian posterior probabilities were obtained from the 50% majority rule consensus of the trees kept as support for nodes.

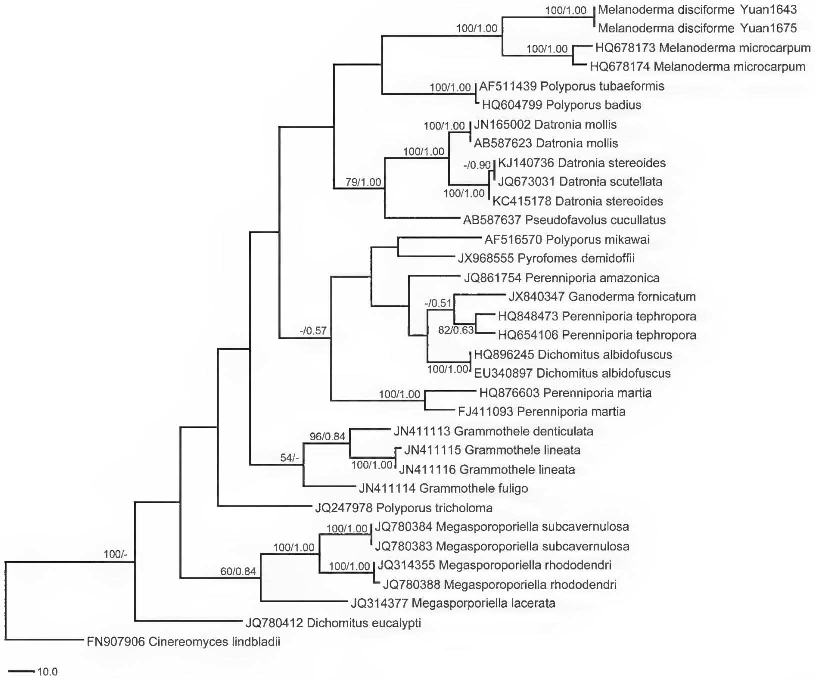
100/1.00
Melanoderma disciforme Yuan1643
Melanoderma disciforme Yuan1675
100/1.00
100/1.00
HQ678173 Melanoderma microcarpum
HQ678174 Melanoderma microcarpum
100/1.00
AF511439 Polyporus tubaeformis
HQ604799 Polyporus badius
100/1.00
JN165002 Datronia mollis
AB587623 Datronia mollis
100/1.00
-/0.90
KJ140736 Datronia stereoides
79/1.00
JQ673031 Datronia scutellata
100/1.00
KC415178 Datronia stereoides
AB587637 Pseudofavolus cucullatus
AF516570 Polyporus mikawai
JX968555 Pyrofomes demidoffii
JQ861754 Perenniporia amazonica
JX840347 Ganoderma fornicatum
-/0.51
HQ848473 Perenniporia tephropora
-/0.57
82/0.63
HQ654106 Perenniporia tephropora
HQ896245 Dichomitus albidofuscus
100/1.00
EU340897 Dichomitus albidofuscus
100/1.00
HQ876603 Perenniporia martia
FJ411093 Perenniporia martia
96/0.84
JN411113 Grammothele denticulata
54/-
JN411115 Grammothele lineata
100/1.00
JN411116 Grammothele lineata
JN411114 Grammothele fuligo
JQ247978 Polyporus tricholoma
100/1.00
JQ780384 Megasporoporiella subcavernulosa
100/-
100/1.00
JQ780383 Megasporoporiella subcavernulosa
60/0.84
100/1.00
JQ314355 Megasporoporiella rhododendri
JQ780388 Megasporoporiella rhododendri
JQ314377 Megasporporiella lacerata
JQ780412 Dichomitus eucalypti
FN907906 Cinereomyces lindbladii
10.0

FIG. 1. Strict consensus tree showing the phylogeny of *Melanoderma disciforme* and related species generated by Maximum Likelihood and Bayesian analysis based on ITS sequences. Parsimony bootstrap values >50% and Bayesian posterior probabilities >0.50 are shown on the branches.

Phylogenetic results

Two ITS sequences were newly generated in this study (GenBank accession numbers KM521268 and KM521269). The ITS dataset contains 34 sequences representing 24 species, among which *Cinereomyces lindbladii* (Berk.) Jülich was selected as outgroup. The alignment comprised 601 characters, of which 297 were constant, 50 were variable but parsimony-uninformative, and 254 were parsimony-informative. Maximum parsimony analysis yielded one parsimonious tree (CI = 0.505, RI = 0.669, RC = 0.338, HI = 0.495). Bayesian analysis ran 5 million generations and resulted in average standard deviation of split frequencies = 0.007737. The 50% majority consensus tree generated by the Bayesian analysis showed a similar topology with the strict consensus MP tree. The strict consensus tree is shown in FIG. 1. Both bootstrap support value (BP) and Bayesian posterior probabilities (BPP) are shown at the nodes. In the phylogenetic tree, two sequences of *M. disciforme* were grouped together with full support (100% BP, 1.00 BPP), and formed a monophyletic lineage with *M. microcarpum* fully supported (100% BP and 1.00 BPP).

Taxonomy

Melanoderma disciforme H.S. Yuan, **sp. nov.** FIG. 2

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> Differs from *Melanoderma microcarpum* by its bigger pores, absence of encrusted cystidia, distinctly smaller cystidioles, and shorter basidiospores.
>
> TYPE: China. Yunnan Prov., Xishuangbanna, Jinghong County, Nabanhe Nat. Res., on fallen angiosperm branch, 15.VIII.2005, Yuan 1675 (**holotype**, IFP; GenBank ITS, KM521269).
>
> ETYMOLOGY: the epithet "*disciforme*" refers to the disk-like basidiocarps.

BASIDIOCARPS: Perennial, effuse-reflexed, growing as round pulvinate patches at first, then fusing laterally, margin narrowly reflexed as a pileus, back attached on substrates, coriaceous when fresh, woody hard upon drying, without odor or taste when fresh; pileus up to 3 mm broad, 5 cm long, and 3 mm thick at base. Pileal surface cream to buff when young, becoming black with a thin crust when aged, concentrically zonate and sulcate, glabrous; margin obtuse, cream to buff, ≤0.5 mm wide. Pore surface white to cream when fresh, buff when dry; pores round to angular, 6–7 per mm, dissepiments thick, entire; sterile margin distinct, ≤0.3 mm wide. Context cream to buff, hard corky, ≤1 mm thick. Tubes cream to buff, corky, distinctly stratified, about 1 mm long for each layer.

HYPHAL STRUCTURE: Hyphal system dimitic; generative hyphae clamped; skeletal hyphae dextrinoid and strongly cyanophilous; tissue unchanged in KOH.

CONTEXT: Generative hyphae scarce, hyaline, thin-walled, rarely branched, 1.5–2.5 µm in diam; skeletal hyphae dominant, hyaline, thick-walled to almost solid, frequently branched, interwoven, 1.5–3 µm in diam.

TUBES: Generative hyphae scarce, hyaline, thin-walled, rarely branched, 2.2–3 µm in diam; skeletal hyphae dominant, hyaline, thick-walled to almost solid, occasionally branched, interwoven, 1–3.5 µm in diam. Cystidia absent; cystidioles fusiform, hyaline, thin-walled, 11–14 × 3–4 µm. Basidia clavate to barrel, with four sterigmata and a basal clamp connection, 10–13 × 5–6 µm; basidioles similar in shape to basidia, but slightly smaller. Polygonal crystals frequently present in trama.

BASIDIOSPORES: Cylindrical, hyaline, thin-walled, smooth, IKI–, CB–, (4.8–)4.9–5.3(–5.5) × (1.9–)2–2.3(–2.5) µm, L = 5.05 µm, W = 2.17 µm, Q = 2.32–2.34 (n = 34/2).

TYPE OF ROT: Causes a white rot in angiosperms.

> ADDITIONAL SPECIMENS EXAMINED: ***Melanoderma disciforme***: **CHINA. YUNNAN PROV.**, Xishuangbanna, Jinghong County, Nabanhe Nat. Res., fallen angiosperm branch, 15.VIII.2005, Yuan 1643 (IFP; GenBank ITS: KM521268).
>
> *Melanoderma microcarpum*: **CHINA. HUNAN PROV.**, Yizhang County, Mangshan Forest Park, on fallen angiosperm trunk, 25.VI.2007, Dai 8116 (BJFC, holotype).

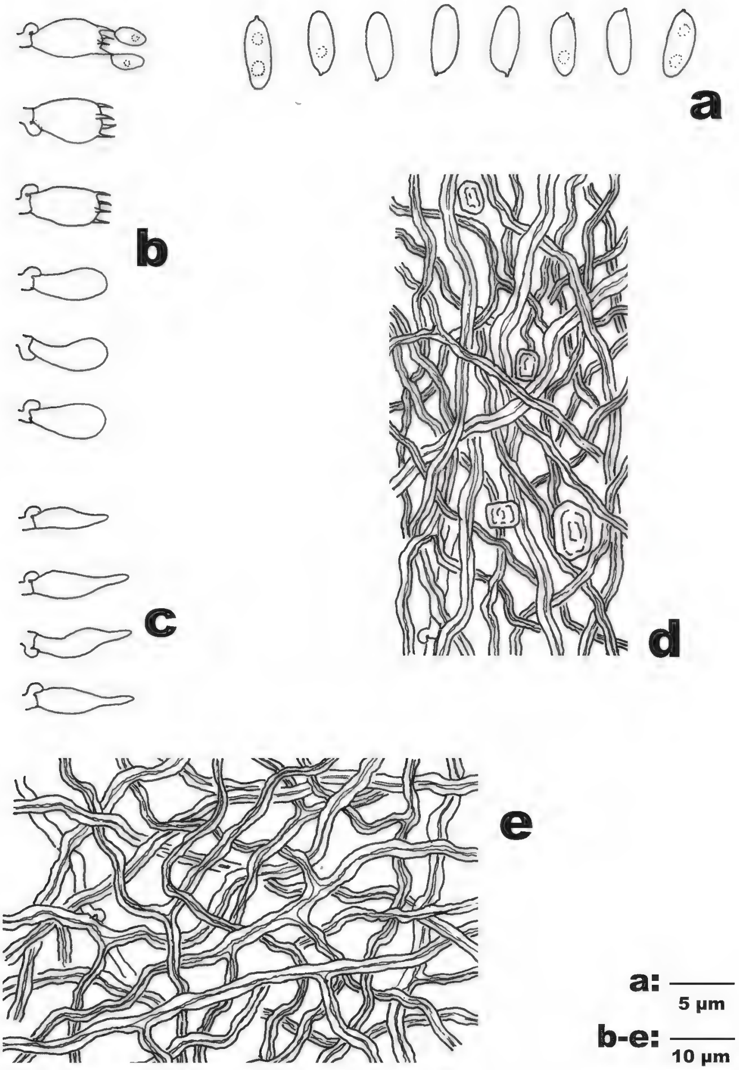

Fig. 2. *Melanoderma disciforme* microscopic structures (Yuan 1675, holotype). a. Basidiospores; b. Basidia and basidioles; c. Cystidioles; d. Hyphae from trama; e. Hyphae from subiculum.

Discussion

Melanoderma disciforme represents the second species in the genus. It is characterized by perennial effuse-reflexed basidiocarps, narrow pileus with a thin black crust on the pileal surface, fusiform cystidioles, and cylindrical basidiospores. The molecular phylogeny and morphological evidences support it as a new species.

Melanoderma disciforme shares most of the morphological features with *M. microcarpum*, the type species of the genus, which differs by smaller pores (7–9 per mm), apically encrusted cystidia, distinctly larger cystidioles, and longer basidiospores.

Species in *Perenniporia*, *Megasporoporiella* B.K. Cui et al., *Datronia* Donk, and *Dichomitus* D.A. Reid are morphologically similar to *M. disciforme* by having a dimitic hyphal system with cyanophilous skeletal hyphae. However, *Perenniporia* species have thick-walled basidiospores, *Megasporoporiella* species lack a crust on the surface of the pileus, and *Datronia* and *Dichomitus* species have non-dextrinoid skeletal hyphae (Núñez & Ryvarden 2001, Li & Cui 2013). Additionally, phylogenetic analyses based on ITS sequences indicate that species sampled from these genera are not genetically close to *M. disciforme*.

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